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Genetic studies on experimental autoimmune gastritis induced by neonatal thymectomy using recombinant inbred strains between a high-incidence strain, BALB/c, and a low-incidence strain, DBA/2

Y. MORI*, M. HOSONO†, K. MURAKAMI*, H. KATOH¶, Y. YOSHIKAWA*, K. KURIBAYASHI*, R. KANNAGI‡, M. SAKAI§, M. OKUMA§ & T. MASUDA* *Department of Immunobiology, Institute for Immunology, Faculty of Medicine, †Department of Immunology, Chest Disease Research Institute, ‡Department of Laboratory Medicine, Faculty of Medicine, and §The First Division, Department of Medicine, Faculty of Medicine, Kyoto University, Kyoto, and ¶Department of Genetics, Central Institute for Experimental Animals, Kawasaki, Japan

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SUMMARY

Thymectomy on day 3 after birth induced autoimmune gastritis (AIG) at the age of 2 months in 51–73% of BALB/c mice, and in only 3–5% of DBA/2 mice. AIG was detected by histological and serological (immunofluorescence staining for detecting anti-parietal cell autoantibody) examination. However, autoantibody was weakly positive in almost all of these DBA/2 mice when measured by ELISA using extract of murine gastric mucosa as the antigen. To investigate genetically the mechanism controlling the incidence of AIG, 11 recombinant inbred strains established by brother–sister mating of (BALB/c × DBA/2) F2 mice (C × D2 strains) were used. Among 26 markers tested, the Mls-1 locus on BALB/c chromosome 1 and the Hc locus coding a complement component (C5) on BALB/c chromosome 2 were found to be associated with high susceptibility to AIG. However, if one or both of the loci were of DBA/2 origin, mice showed medium or low susceptibility to AIG. For further analysis, F1, F2 and back-cross generations of these two strains were tested, but segregation of a single susceptibility or insusceptibility gene was not obtained. Taken together, it seems probable that two or more genes are involved in the induction mechanism of AIG. We did not detect C5 deposition in AIG lesions, nor complement-dependent cytotoxic antibody to parietal cells in serum from AIG mice. However, injection of irradiated spleen cells of DBA/2 mice into BALB/c mice thymectomized on day 3 augmented the incidence of AIG from 71 to 100%, but not that of oophoritis (33%). A relationship between Mls-1^a determinants and the pathogenesis of AIG was further suggested from the fact that $V_{\beta 6}$ TcR-expressing T cells increased in number in AIG-bearing compared with normal BALB/c mice.

Keywords murine gastritis thymectomy autoimmune diseases T cell receptors Mls

INTRODUCTION

Administration of antigens, such as thyroglobulin (Rose & Witebsky, 1956), myelin basic protein (Weigle, 1980), acetylcholine receptor (Lindstrom, Shelton & Fujii, 1988), and collagen (Trentham, Thomas & Kang, 1977) is known to induce autoimmune phenomena in various animals. Imbalances of the immune system created by, for example, neonatal thymectomy on day 3 (Nishizuka & Sakakura, 1969; Kojima & Prehn, 1981), can also produce an autoimmune state in mice. This is manifested by organ-specific effects, such as gastritis, oophoritis, thyroiditis, or orchitis, and is accompanied by the production of autoantibodies to innate autoantigens of a number of organs. Autoimmune thyroiditis also develops in adult rats that have undergone thymectomy followed by repeated doses of sublethal irradiation (Penhale *et al.*, 1973).

We have investigated the induction mechanisms of autoimmune diseases using day-3-thymectomized mice (Sakaguchi *et al.*, 1985; Fukuma *et al.*, 1988; Mori *et al.*, 1989) and demonstrated that a T cell subset expressing Lyt1 antigen may play a key role in the induction of tissue damage as an effector of delayed-type hypersensitivity (DTH) (Fukuma *et al.*, 1988). Interestingly, a Lyt1 subset also acts as a suppressor or suppressor-inducer for the maintenance of self tolerance to autoantigens by inactivating effector T cells (Sakaguchi *et al.*,

Correspondence: Tohru Masuda, Department of Immunobiology, Institute for Immunology, Faculty of Medicine, Kyoto University, Yoshida, Sakyo, 606 Kyoto, Japan.

1985). The mechanisms underlying the development and maintenance of T cell tolerance to self antigens are not clear, but are generally thought to fit one or both of two models: functional deletion or the suppression of self-reactive clones. Our findings have indicated that the latter mechanism is in operation in our murine model.

Kojima & Prehn (1981) have reported that various susceptibility genes, including the H-2 complex, may be involved in the development of localized autoimmune diseases in day-3-thymectomized mice. With regard to autoimmune gastritis (AIG), the susceptibility of BALB/c mice is high, while that of H-2-matched DBA/2 mice is low. This suggests that there is actually no association with MHC antigens. These two strains of mice carry different minor lymphocyte-stimulating (Mls) determinants: Mls-1^b for BALB/c mice and Mls-1^a for DBA/2 mice (Festenstein, 1974; Janeway, Fischer-Lindahl & Hammerling, 1988).

We have recently established recombinant inbred strains of mice termed C × D2 (Gyotoku *et al.*, 1989) from F2 of BALB/cCrSlc and DBA/2CrSlc strains of mice by repeated brother–sister mating. Here we used these strains in addition to BALB/c and DBA/2 mice to examine inheritance of AIG traits, which are possibly not linked to the MHC. We demonstrated that at least a combination of the Mls-1 locus and the Hc locus for C5 (Erickson *et al.*, 1964) was associated with susceptibility to AIG, although involvement of additional genetic factors could not be ruled out.

MATERIALS AND METHODS

Mice

Male and female BALB/cCrSlc and DBA/2CrSlc were maintained in the Facility of Experimental Animals at the Faculty of Medicine of Kyoto University under specific pathogen-free conditions. F1, F2 and backcross generations between these two strains were produced in the same facility. Fifteen recombinant inbred strains descended from F2 of these two strains termed C × D2 RI strains were established (Gyotoku *et al.*, 1989). These mice were maintained in the Animal Facility of the Chest Disease Research Institute of Kyoto University.

Genetic monitoring

All 11 strains as well as the original parental strains of BALB/c and DBA/2 mice underwent genetic monitoring for 26 loci, as described elsewhere (Nomura, Esaki & Tomita, 1984). The following markers were analysed: four morphological markers (a, b, c and d), eight immunological markers by mixed lymphocyte reactions for Mls-1, by Ouchterlony's test in gel using antibody for Hc, by immunofluorescence staining using relevant antibodies for $V_{\beta 6}$–TcR, Lyt1, Lyt2, and Thy1.2 (see below), and by complement-dependent cytotoxic tests using relevant monoclonal antibodies (MoAbs) and rabbit complement for H-2K and D, 11 enzymatic markers by electrophoresis on Titan III cellulose acetate plates (Helena Lab., Beaumount, TX) for Idh-1, Pep-3, Pgm-1, Gpi-1, Car-2, Gpd-1, Es-1, Es-2, Mod-1, and Es-3, and on isoelectric focusing gel for Es-10, and four protein markers by electrophoresis on 10% polyacrylamide gel for Svp-1, Mup-1, Hba, and Trf.

Thymectomy

On day 3 after birth, mice were thymectomized as described previously (Sakaguchi *et al.*, 1985). The completeness of the thymectomy was confirmed when mice were killed.

Immunohistochemical examinations

Murine sera were obtained from 4- to 12-week-old thymectomized or normal BALB/c mice. Indirect immunofluorescence (IF) staining with murine serum was performed using 4-μm sections of neutral-buffered formalin-fixed and polyester wax-embedded specimens, as described elsewhere (Sakaguchi *et al.*, 1985). Control staining was carried out using normal mouse serum in place of the first autoantibodies. The second antibody was FITC-labelled rabbit anti-mouse immunoglobulin purchased from Cappel Products (Westchester, PA).

Flow cytometry

In order to prevent FcR-mediated binding of the primary monoclonal antibody, lymphocytes separated from either inguinal and axillary lymph nodes of normal BALB/c mice or small curvatural lymph nodes of AIG-bearing BALB/c mice were suspended in Eagle's minimum essential medium (MEM) containing 0·5% normal mouse serum. One-million cells each were then incubated at 4°C for 30 min with FITC-labelled MoAbs, such as anti-Thy1.2 (mouse IgG2b; Caltag, South San Francisco, CA), anti-L3T4 (rat IgG, GK-1·5), or anti-Lyt2 (rat IgG2a, Becton Dickinson, Mountain View, CA). An equal number of cells was also incubated under the same condition with 25 μl of culture supernatant of hybridoma cell line cells, 44-22-1 (rat IgG2b, anti-$V_{\beta 6}$), or biotinylated anti-$V_{8\cdot1/8\cdot2}$ rat IgG2b, F-23.1) followed by further incubation with FITC-labelled mouse anti-rat IgG (Cappel Lab., Malvern, PA) or phycoerythrin-labelled streptavidin (Becton Dickinson). The staining conditions were determined by either the direction of the supplier or preliminary tests, and percentage of each cells stained were determined using a flow cytometer (FACScan, Becton Dickinson). The primary antibody was omitted for a negative control, in the case of TcR staining. The results were expressed as the ratios of each T cell subsets to total Thy1.2-positive cells in normal and AIG-bearing mice, respectively. The significance of differences between these two groups was calculated by Student's *t*-test.

Preparation of gastric extract

The gastric extract used for ELISA was prepared as reported previously (Mori *et al.*, 1989). Briefly, fresh murine gastric mucosa (from which the squamous epithelium and antral portions had been removed) was collected by scraping with a glass slide, and then homogenized in 4 vol of homogenizing buffer (10 mM Tris–HCl (pH 7·4), 1 mM $MgCl_2$, and 0·25 M sucrose) using a Tenbroeck tissue grinder (Wheaton, Millville, NJ). The homogenate was then further sonicated with a sonicator (Ultrasonics, Plainview, NY) for 5 min and then centrifuged at 7000 ***g*** for 30 min. The supernatant was next ultracentrifuged at 100 000 ***g*** for 100 min and the resulting pellet (the whole microsomal fraction) was suspended in a homogenizing buffer after a single washing. The protein concentration was estimated using the Protein assay kit (Bio-Rad Laboratories, Richmond, CA), with bovine serum albumin as the standard. All procedures were performed at 4°C.

ELISA

Flat-bottomed polyvinyl microtitre plates (96 wells; Falcon, Becton Dickinson), were coated overnight at 4°C with 100-μl aliquots of the whole microsomal fraction (20 μg/ml) diluted in phosphate-buffered saline (PBS). After blocking non-specific binding with PBS containing 1% bovine serum albumin (Armour, Kankakee, IL), the wells were washed three times with 250 μl of PBS containing 0·05% Tween 20.

For titration of autoantibodies, 50-μl aliquots of serially diluted sera (1/100 to 1/6,400) were plated and incubated for 1 h followed by three washes. Subsequently, 50 μl of peroxidase-conjugated goat anti-mouse IgG (heavy and light chain specific; Cappel) diluted to 1/1000 were added. After 1 h, the plates were washed five times. One-hundred microlitres of substrate solution containing *o*-phenylenediamine were then added and incubation was performed for 15 min. The reaction was terminated using 25 μl of 2 M H_2SO_4 and the absorbance at 500 nm was determined with an ELISA plate reader (MTP-12 microplate photometer; Corona, Tokyo, Japan).

^{51}Cr release assay

One-hundred and fifty microlitres of ^{51}Cr (Japan Atomic Energy Research Institute, Tokyo, Japan) were mixed with 1×10^6 murine stomach cells isolated by the method previously described (Fukuma *et al.*, 1988) and then incubated for 1 h at 37°C. After washing three times, the cells were suspended in MEM containing 10% fetal calf serum (FCS, Flow Lab., McLean, VA) at a concentration of 1×10^6/ml. Then 0·1-ml aliquots were incubated with equal volumes of pooled AIG mouse serum and guinea pig complement (both appropriately diluted) for 30 min at 37°C in a 96-well flat-bottomed culture plate (Nunc, Roskilde, Denmark). The radioactivity in a 100-μl volume of the supernatant was measured by a gamma counter (Packard), and per cent cytotoxicity was calculated according to the formula:

$$\frac{\text{Experimental} - \text{Spontaneous release}}{\text{Total} - \text{Spontaneous release}} \times 100\%$$

Immunofluorescence staining for C5

Frozen sections of AIG stomachs were fixed with cold acetone for 10 min. After washing with PBS, sections were incubated with appropriately diluted polyclonal rabbit anti-mouse C5 antiserum (kindly presented by Dr N. Tamura, Tsukuba University, Japan) for 30 min at room temperature, and then stained with FITC-labelled sheep anti-rabbit IgG antibody for 30 min.

Spleen cells

Cell suspensions from either DBA/2 or BALB/c spleens were prepared in MEM without FCS, as described elsewhere (Hosono, Hosokawa & Katsura, 1986) and then treated with appropriately diluted anti-Thy1.2 MoAb (Meiji Institute of Health Science, Tokyo, Japan) for 40 min, followed by incubation at 37°C for 30 min in the presence of 1/15 diluted normal rabbit serum as a source of complement (kindly provided by Dr T. Hosokawa, Kyoto Prefectural Medical School). After washing, the cells were irradiated at 20 Gy.

Cell transfer

Ten-million irradiated splenic cells (25 μl) of either DBA/2 or BALB/c mice were injected into both foot-pads of BALB/c mice who had been thymectomized on day 3 of age 1 month previously. The mice were examined histologically and serologically at the age of 3 months. Age-matched normal BALB/c mice were also injected with the irradiated DBA/2 spleen cells as a control.

Table 1. Incidence of post-thymectomy autoimmune gastritis in BALB/c and DBA/2 mice

Strain	Mice with autoimmune gastritis/total thymectomized mice (%)		
	Female	Male	Total
BALB/c (H-2^d)	14/27 (52)	6/12 (50)	20/39 (51)
DBA/2 (H-2^d)	1/18 (6)	0/18 (0)	1/36 (3)

Mice were thymectomized at 3 days of age, and histological and serological examinations were made after 2 months.

Histological examination

Stomach and other tissues (thyroid, ovary and testis) were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with haematoxylin and eosin. Assessment was made by an investigator who had no prior knowledge of the origin of the sections.

RESULTS

Inheritance of susceptibility to AIG in BALB/c and DBA/2 mice

The incidence of post-thymectomy AIG in BALB/c and DBA/2 mice was determined in the first experiment. As shown in Table 1, 51% of BALB/c mice that underwent thymectomy on day 3 after birth had developed AIG in association with the production of anti-parietal cell autoantibody when examined serologically and histologically 2 months after birth. In contrast, DBA/2 mice were resistant to AIG, with only one out of 36 mice (3%) developing gastritis. There was no sex difference in the incidence of AIG and neither a 100% nor a 0% incidence of AIG was attained by day-3 thymectomy in BALB/c or DBA/2 mice.

BALB/c mice that produced the autoantibody, as detected by indirect IF staining of normal stomachs, always developed AIG lesions accompanied by infiltration of mononuclear cells into the gastric mucosa and vice versa (Fig. 1). Serum with a high ELISA titre was always positive by IF staining (Fig. 2a, b, bold lines) and such mice always manifested AIG lesions. However, neither AIG lesion nor autoantibody detectable by IF staining was found in some BALB/c mice (Fig. 2a, b, fine lines). Only one DBA/2 mouse was serologically and histologically positive. The others developed no AIG lesions or autoantibody detectable by IF staining. Nevertheless, antibody titres detected by ELISA were weakly positive, as shown in Fig. 2(c, d). Sera of age-matched, normal either BALB/c or DBA/2 mice were negative in ELISA in addition to IF staining (data not shown).

Incidence of AIG in recombinant inbred C × D2 strains

Four out of 15 recombinant inbred strains were excluded from the present study, since the numbers of mice were insufficient for evaluation. The remaining 11 strains tested were arbitrarily

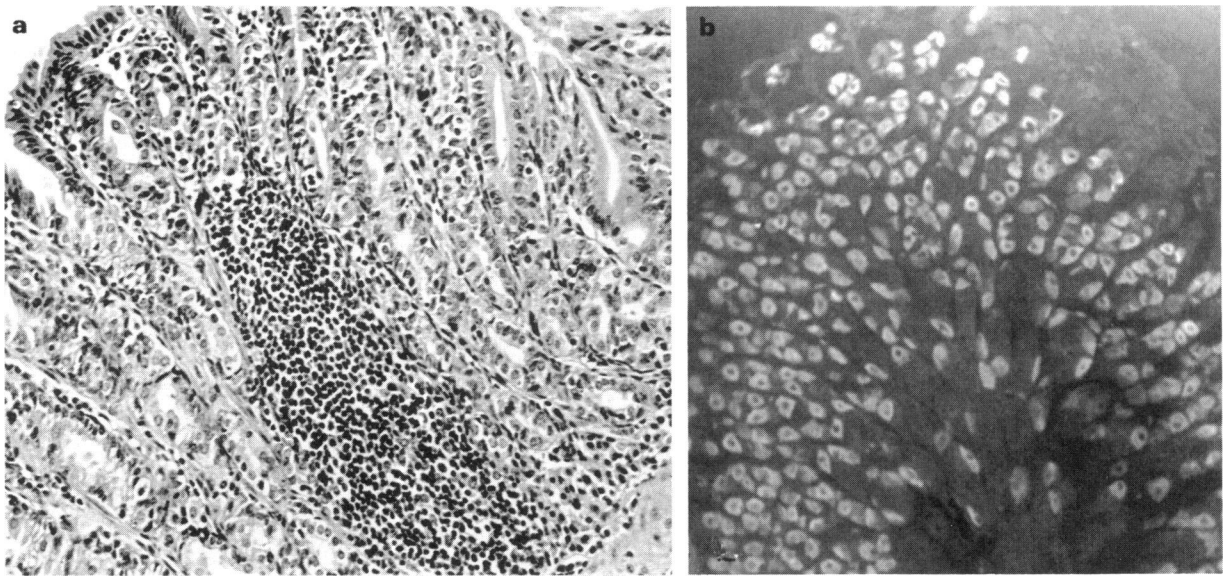

Fig. 1. (a) Histological appearance of gastric mucosa of a mouse with autoimmune gastritis (AIG). Inflammatory cells, mainly mononuclear cells, are infiltrating the submucosa and epithelium; (b) indirect immunofluorescence staining of normal gastric mucosa using serum of a BALB/c mouse with AIG, 3 months after thymectomy at 3 days of age. Original magnification, × 100.

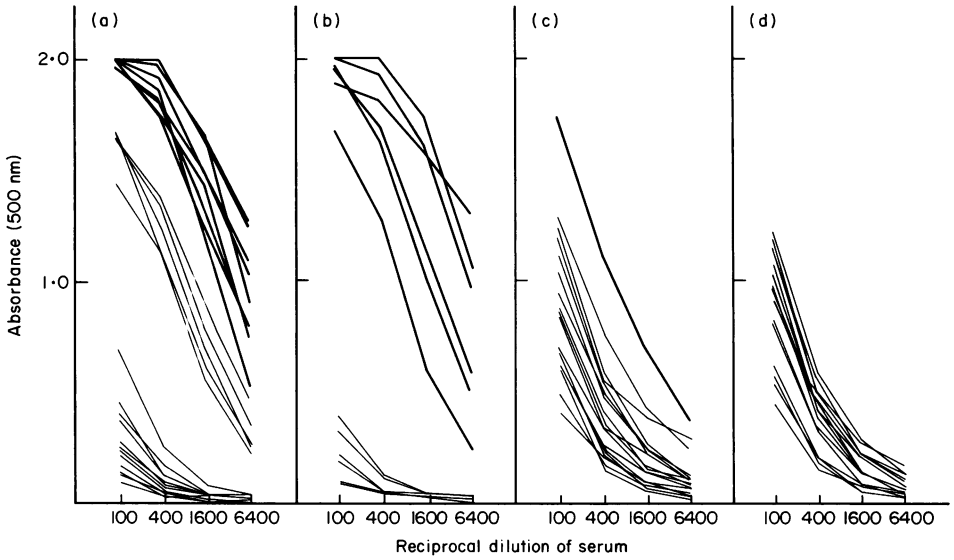

Fig. 2. Serum anti-parietal cell autoantibody titres measured by ELISA (using extract of murine gastric mucosa as the antigen) in female (a) and male (b) BALB/c and female (c) and male (d) DBA/2 mice that had been thymectomized at 3 days of age 2 months previously. Bold lines, histologically positive for autoimmune gastritis lesions and immunofluorescence staining positive. Fine lines, both negative.

divided into three groups on the basis of the incidence of AIG determined by histological examination and IF staining: a high incidence group (H) including four strains (26–47%); a medium incidence group (M) with three strains (13–17%); and a low incidence group (L) with four strains (4–7%) (Table 2). Figures 3 and 4 demonstrate that the results obtained by histological findings and IF staining coincided well with those obtained by ELISA (two H strains, C × D2-6 and C × D2-13, and two L strains, C × D2-3 and C × D2-10). No antibodies to parietal cells were detected serologically in sera of the unthymectomized H strain mice, and there was no significant difference in incidence between males and females in these strains.

It was clearly demonstrated that H strains always carried both the Mls-1 locus on chromosome 1 and the Hc locus on chromosome 2 of BALB/c origin as a pair, while other strains possessed either or both loci of DBA/2 origin. It was also noteworthy that T cells expressing $V_{\beta 6}$ TcR were detected in the lymph nodes of normal (unthymectomized) H strain (including BALB/c) mice, although $V_{\beta 8}$–TcR expressing cells existed in both H and L strains (data not shown). Thus, it was suggested

Table 2. Genetic profile of C × D2 strains and incidence of autoimmune gastritis (AIG)

	Chromosome no. and locus														Incidence of AIG*		
	1			2			5	6	7	9	11		14	19			
Strain	Idn-1	Pep-3	Mls-1	HC	a	Svp-1	Pgm-1	Lyt.2	c	d	Hba	Es-3	Es-10	Lyt.1		%	*n*
BALB/c:C	a	a	b	1	A	b	a	b	c	D	c	a	a	b	H	51	39
DBA/2:D	b	b	a	0	a	a	b	a	C	d	g	c	b	a	L	3	36
C × D2-1	D	C	C	C	D	C	D	D	D	D	D	D	C	D	H	26	47
C × D2-2	D	C	D	C	D	D	D	D	C	D	D	D	C	D	M	17	24
C × D2-3	D	D	D	D	C	C	C	D	C	C	C	C	D	C	L	4	53
C × D2-5	D	C	C	C	D	D	D	D	D	D	D	D	C	D	H	27	30
C × D2-6	D	C	C	C	D	D	D	D	D	D	D	D	C	D	H	33	40
C × D2-7	D	D	C	D	C	C	C	D	D	C	C	C	C	D	M	13	31
C × D2-8	D	D	D	C	C	C	D	C	C	C	C	C	D	C	L	4	49
C × D2-10	D	D	D	C	C	C	D	D	D	C	C	C	D	C	L	5	40
C × D2-11	D	D	D	D	C	C	C	D	C	C	C	C	D	D	M	13	38
C × D2-13	D	D	C	C	C	C	D	D	C	D	C	D	D	D	H	47	32
C × D2-14	D	D	C	D	D	D	C	D	D	D	C	C	D	D	L	7	45

In the C × D2 series, BALB/c and DBA/2 alleles are indicated by C and D, respectively.
* Determined by histological and serological examinations 2 months after thymectomy at 3 days of age.
H, high incidence; M, medium incidence; L, low incidence.

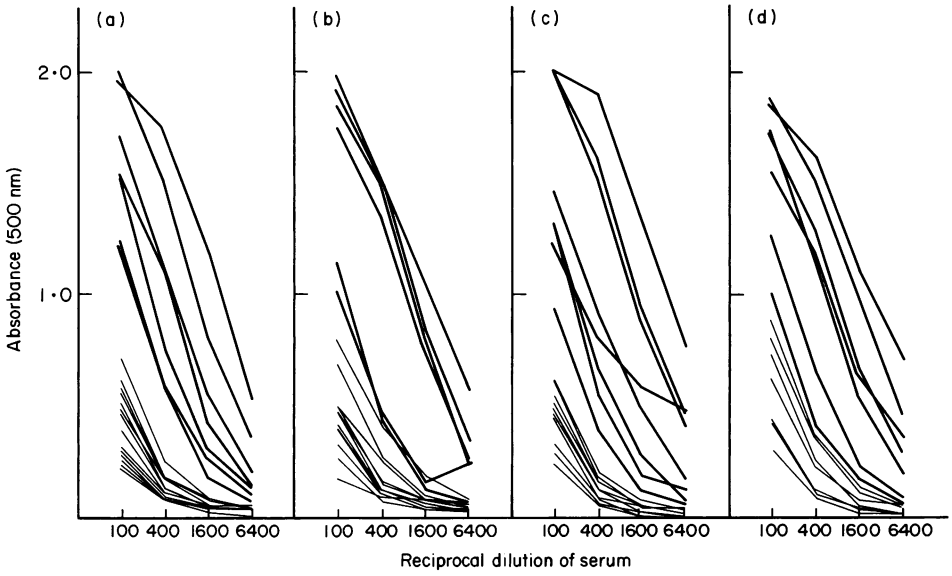

Fig. 3. Serum titres of anti-parietal cell autoantibodies measured by ELISA (using extract of murine gastric mucosa) in female (a, c) and male (b, d) H-recombinant inbred strains, C × D2-6 (a, b) and C × D2-13 (c, d).

from this genetic analysis that at least the genes for Mls determinants and complement C5 may associate with the mechanism causing AIG. On the basis of these findings, we next investigated the genetic trait of postulated susceptibility gene(s) for AIG using (BALB/c × DBA/2) and (DBA/2 × BALB/c) F1, F2 and the back-crossed mice. As expected, segregation of a single gene controlling the induction of AIG was not obtained, although a trend was observed that, in addition to other undetermined genes, DBA/2 mice may express an insusceptibility gene which is dominant to a susceptibility gene in BALB/c mice (data not shown).

Booster effect of Mls-1^a determinants on the development of AIG in BALB/c mice

To investigate whether AIG increased in incidence in BALB/c mice when responder T cells for Mls-1^a determinants were activated, BALB/c mice thymectomized on day 3 were challenged with Mls-1^a spleen cells 1 month after birth and then killed at the age of 2 months. It is shown in Table 3 that the 73% incidence of AIG in the original day-3-thymectomized mice was augmented to virtually 100% by challenge with Mls-1^a cells. No enhancement of AIG induction is caused by injection of DBA/2 spleen cells into euthymic BALB/c mice or BALB/c spleen cells

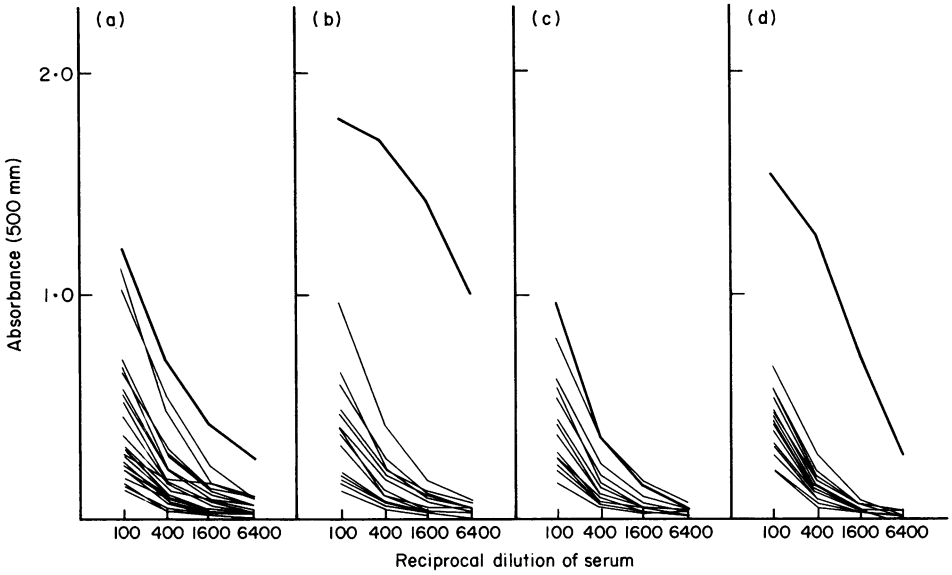

Fig. 4. Serum titres of anti-parietal cell autoantibodies measured by ELISA (using extract of murine gastric mucosa) in female (a, c) and male (b, d) L-recombinant inbred strains, C × D2-3 (a, b) and C × D2-10 (c, d).

Table 3. Booster effect of Mls-1^a (DBA/2) cells on autoimmune gastritis (AIG) induction in Mls-1^b (BALB/c) mice thymectomized at 3 days of age

Cells*	Thymectomy	Incidence (%)† AIG	AIO	Mice (*n*)
Mls-1^a	−	0	0	7
Mls-1^b	+	66	NT	6
—	+	73	30	26
Mls-1^a	+	100	33	12

* Splenic B cells were injected into the foot-pad 1 month after thymectomy.

† Determined serologically and histologically 2 months after thymectomy.

AIO, autoimmune oophoritis; NT, not tested.

Table 4. Relative increase in $V_{\beta 6}$–TcR-expressing cells in BALB/c mice with autoimmune gastritis (AIG)

T cell markers	Positive cells (%)* Normal mice†	AIG mice‡
Thy1.2	68 ± 3	24 ± 6
L3T4	53 ± 2 (76 ± 3)	19 ± 5 (82 ± 4)
Lyt2.2	18 ± 2 (27 ± 3)	8 ± 2 (33 ± 5)
$V_{\beta 6}$	8 ± 0 (11 ± 1)§	4 ± 1 (16 ± 1)§
$V_{\beta 8}$	22 ± 2 (32 ± 2)	7 ± 2 (28 ± 5)

* Numbers in parentheses indicate proportions of each subset in the total Thy1.2-positive cells.

† Peripheral (inguinal and axillary) lymph node cells.

‡ Small curvetural lymph nodes.

§ $0{\cdot}01 < P < 0{\cdot}001$ between normal and AIG mice.

depleted of T cells by anti-Thy1.2+C treatment into day-3-thymectomized BALB/c mice. Moreover, it is important to note that the incidence of oophoritis (33%) was never concomitantly augmented by injection of Mls-1^a cells into these two experiments, implying that the booster effect of Mls-1^a cells was AIG specific.

Increase of $V_{\beta 6}$-expressing T cells in AIG-bearing BALB/c mice

Usually, small curvetural lymph nodes which are regional nodes of stomachs are enlarged in AIG-bearing mice, in contrast with those of normal mice which are too small to use for experiments. Distribution of T cell subsets was examined in these regional lymph nodes of AIG mice compared with that in peripheral (inguinal and axillary) lymph nodes of age-matched normal BALB/c mice. The results obtained by flow cytometic analysis of these lymph node cells after staining with MoAbs are summarized in Table 4. Thy1.2-positive cells were 68% in normal, and 24% in AIG mice, reflecting the influence of thymectomy at day 3. A low percentage was also observed for T cells expressing L3T4, Lyt2, $V_{\beta 6}$ and $V_{\beta 8}$. Therefore, the percentage of each subset within the Thy1.2-positive cell population in the lymph nodes of AIG mice was compared with that of normal mice. As shown in parentheses, relative increase in number was significant only in the case of $V_{\beta 6}$–TcR-positive cells. Although not statistically significant, L3T4$^+$ and Lyt2.2$^+$ cells increased, whereas $V_{\beta 8}$–TcR$^+$ cells decreased slightly in number in AIG compared with normal mice.

Lack of complement dependency of anti-parietal cell autoantibody activity

The final experiment was carried out to determine whether C5 was responsible for tissue damage or not. Parietal cells were labelled with ^{51}Cr, and subjected to complement-dependent

cytolysis using guinea pig complement and pooled serum of AIG mice, containing polyvalent anti-parietal cell autoantibody. No significant level of ^{51}Cr release was attained with either 1/20 or 1/10 diluted sera, indicating no significant cell killing (data not shown). Moreover, no deposition of C5 in the AIG stomachs was detected by indirect IF staining using rabbit anti-mouse C5 and FITC-labelled sheep anti-rabbit IgG antibody. Thus, direct involvement of C5 in the pathogenesis of AIG is unlikely.

DISCUSSION

We have shown that H strains for post-thymectomy murine AIG carried at least two BALB/c loci, Mls-1 on chromosome 1 and Hc on chromosome 2. When one or both of them were DBA/2 loci, the incidence was medium or low. However, genetic analysis using F1, F2, and back-cross generations bred from BALB/c and DBA/2 mice resulted in no clear segregation of either dominant or recessive genes. This suggests that at least two or more genes could be involved in the induction mechanism of AIG. Kojima & Prehn (1981) reported on the basis of experiments using recombinant inbred strains descended from BALB/c mice (high incidence of AIG) and C57B1/6 mice (low incidence of AIG), that susceptibility to AIG was not associated with MHC determinants but appeared to be influenced by a minor histocompatibility locus, perhaps the H-27 locus on chromosome 5. Although H-27 was not investigated in our study, both the BALB/c and C57B1/6 strains also bear Mls-1^b. Their results appear to suggest that Mls-1 is not always involved directly in susceptibility to AIG, in contrast to our observations. This discrepancy can not be explained at present. In addition, the finding that neither a 100% nor a 0% incidence of AIG was ever achieved by thymectomy also suggests genetic traits of post-thymectomy autoimmune diseases (including AIG) are quite complex and may have multi-factorial control: M strains, in which no consistent results on the incidence of AIG (Table 2) and expression of $V_{\beta 6}$–TcR expression (data not shown) were obtained, could be the case, and unknown environmental factors may be involved in the development of AIG. Despite this problem, the possible linkage of Hc and Mls genes with AIG in the murine model as shown in H strains appears to deserve strong consideration.

The indirect association of complement with the development of some autoimmune diseases has been suggested (Rosenfield, Kelly & Leddy, 1976), but the extent of active involvement of the complement system in their pathogenesis is unknown. Although the Hc locus coding complement C5 was apparently linked with susceptibility to post-thymectomy AIG in combination with the Mls locus, we failed to find any role for C5 in the mechanism of tissue damage. There was no complement-dependent cytotoxicity directed towards parietal cells and no C5 deposition in AIG stomachs. Thus, undetermined loci near Hc may be linked with susceptibility to AIG.

In most serum sample from DBA/2 mice that underwent day-3 thymectomy, low but still significant levels of autoantibodies were detected, despite the fact that these mice developed no AIG lesions. In addition, AIG serum never transfers AIG in normal mice (Fukuma *et al.*, 1988) and normal T cells of syngeneic mice can prevent the appearance of autoimmune diseases (Sakaguchi, Takahashi & Nishizuka, 1982a, 1982b). Taken together, it is conceivable that T cells, but not autoantibody play an essential role in the induction of post-thymectomy autoimmune diseases.

Thus, the detection of a linkage with the Mls-1 locus sheds new light on the pathogenesis of autoimmune diseases. Mls determinants, which have been found only in mice at present, are non-MHC gene products expressed by antigen-presenting cells including B cells (Sunshine *et al.*, 1985; Webb *et al.*, 1989; Molina *et al.*, 1989). A very high proportion of T cells from Mls-1^b mice shows the ability to respond to leucocytes expressing the Mls-1^a (Festenstein, Kumura & Biasi, 1989). It has been postulated that the TcR repertoire is shaped by both negative selection (Nossal, 1983) and positive selection (Zinkernagel & Doherty, 1979) in association with MHC antigens, although the cellular and molecular basis of repertoire selection remains unclear. It has been demonstrated that $V_{\beta 6}$ (Kappler *et al.*, 1988; Schneider *et al.*, 1989) and $V_{\beta 8.1}$ (MacDonald *et al.*, 1988b) TcR expression are strongly associated with T cell recognition of Mls-1^a determinants. Moreover, these determinants apparently play the role of ligands in the elimination of self-reactive/$V_{\beta 6}$- or $V_{\beta 8.1}$-expressing T cells in Mls-1^a-positive strains. In BALB/c mice rendered tolerant for Mls-1^a determinants by the injection of DBA/2 spleen cells within 24 h of birth, it was found that $V_{\beta 6}$ TcR-expressing cells were also eliminated (MacDonald *et al.*, 1988a, 1989). Hence, it is important that we found that $V_{\beta 6}$ TcR-expressing T cells existed in unthymectomized H but not in L strains of mice. Moreover, we observed a significant increase in the proportion of $V_{\beta 6}$ TcR-expressing T cells in the regional lymph nodes of AIG BALB/c mice. Such cells are thought to respond to Mls-1^a determinants. Furthermore, 13 out of 20 T cell clones established from T cells obtained from the mucosa and regional lymph nodes of AIG stomachs using parietal cells as the antigen carried $V_{\beta 6}$ TcR and produced interferon-gamma (manuscript in preparation). These observations all suggest an intimate relationship between Mls-1^a determinants and AIG.

The specific augmentation of post-thymectomy AIG but not of oophoritis in BALB/c mice injected with Mls-1^a cells reflected promotion of the activity of effector T cells causing tissue damage by Mls-1^a determinants. At present, two explanation for this booster effect of Mls-1^a cells on AIG induction are conceivable. One is that Mls-1^a antigens may stimulate particular T cell clones including $V_{\beta 6}$-expressing cells, thereby concomitantly activating the effector to cause AIG. Another is that parietal cells may share a common epitope with Mls-1^a antigens that also share antigenicity with the product of pathogenic bacteria (Janeway *et al.*, 1989). Further studies to clarify these possibilities are in progress.

Whatever the final explanation, the murine model described here should facilitate analysis of the cellular and molecular events associated with the pathogenesis of autoimmune diseases.

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